

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

Food and Drug Administration Papers

U.S. Department of Health and Human Services

2017

# Cannabinoid disposition in oral fluid after controlled smoked, vaporized, and oral cannabis administration

Madeleine J. Swortwood

*National Institutes of Health*

Matthew N. Newmeyer

*University of Maryland at Baltimore*

Maria Andersson

*National Institutes of Health*

Osama A. Abulseoud

*National Institutes of Health*

Karl B. Scheidweiler

*National Institutes of Health*, [kscheidweiler@intra.nida.nih.gov](mailto:kscheidweiler@intra.nida.nih.gov)

*See next page for additional authors*

Follow this and additional works at: <http://digitalcommons.unl.edu/usfda>



Part of the [Dietetics and Clinical Nutrition Commons](#), [Health and Medical Administration Commons](#), [Health Services Administration Commons](#), [Pharmaceutical Preparations Commons](#), and the [Pharmacy Administration, Policy and Regulation Commons](#)

Swortwood, Madeleine J.; Newmeyer, Matthew N.; Andersson, Maria; Abulseoud, Osama A.; Scheidweiler, Karl B.; and Huestis, Marilyn A., "Cannabinoid disposition in oral fluid after controlled smoked, vaporized, and oral cannabis administration" (2017). *Food and Drug Administration Papers*. 9.  
<http://digitalcommons.unl.edu/usfda/9>

This Article is brought to you for free and open access by the U.S. Department of Health and Human Services at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Food and Drug Administration Papers by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

---

**Authors**

Madeleine J. Swortwood, Matthew N. Newmeyer, Maria Andersson, Osama A. Abulseoud, Karl B. Scheidweiler, and Marilyn A. Huestis

# Cannabinoid disposition in oral fluid after controlled smoked, vaporized, and oral cannabis administration

Madeleine J. Swortwood,<sup>a</sup> Matthew N. Newmeyer,<sup>a,b</sup> Maria Andersson,<sup>a</sup> Osama A. Abulseoud,<sup>a</sup> Karl B. Scheidweiler<sup>a\*</sup> and Marilyn A. Huestis<sup>a,c</sup>

Oral fluid (OF) is an important matrix for monitoring drugs. Smoking cannabis is common, but vaporization and edible consumption also are popular. OF pharmacokinetics are available for controlled smoked cannabis, but few data exist for vaporized and oral routes. Frequent and occasional cannabis smokers were recruited as participants for four dosing sessions including one active (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC) or placebo cannabis-containing brownie, followed by one active or placebo cigarette, or one active or placebo vaporized cannabis dose. Only one active dose was administered per session. OF was collected before and up to 54 (occasional) or 72 (frequent) h after dosing from cannabis smokers. THC, 11-hydroxy-THC (11-OH-THC), 11-nor-9-carboxy-THC (THCCOOH), tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabigerol (CBG) were quantified by liquid chromatography-tandem mass spectrometry. OF cannabinoid  $C_{\max}$  occurred during or immediately after cannabis consumption due to oral mucosa contamination. Significantly greater THC  $C_{\max}$  and significantly later THCV, CBD, and CBG  $t_{\text{last}}$  were observed after smoked and vaporized cannabis compared to oral cannabis in frequent smokers only. No significant differences in THC, 11-OH-THC, THCV, CBD, or CBG  $t_{\max}$  between routes were observed for either group. For occasional smokers, more 11-OH-THC and THCCOOH-positive specimens were observed after oral dosing than after inhaled routes, increasing % positive cannabinoid results and widening metabolite detection windows after oral cannabis consumption. Utilizing 0.3  $\mu\text{g/L}$  THCV and CBG cut-offs resulted in detection windows indicative of recent cannabis intake. OF pharmacokinetics after high potency CBD cannabis are not yet available precluding its use currently as a marker of recent use. Published 2016. This article is a U.S. Government work and is in the public domain in the USA.

**Keywords:** cannabinoids; oral fluid; smoking; vaporizer; edibles

## Introduction

Cannabis remains the most commonly used illicit drug worldwide.<sup>[1]</sup> The main psychoactive compound in cannabis,  $\Delta^9$ -tetrahydrocannabinol (THC), was detected in 12.6% of US week-end night-time drivers' blood or oral fluid (OF) samples<sup>[2]</sup>; increased crash risk is associated with cannabis intake.<sup>[3–6]</sup> OF is an important matrix for detecting drugs of abuse, particularly in driving under the influence of drugs (DUID) testing programmes.<sup>[7–13]</sup> OF collection is advantageous over urine and blood, as it is collected under direct observation, deterring adulteration, without requiring specialized collection by medical personnel. Inhalation via smoking is the most common cannabis administration route, although inhalation via vaporization and oral consumption via edibles frequently occurs.<sup>[14]</sup> To date, data are available from a few OF cannabinoid disposition studies following controlled smoked cannabis<sup>[15–20]</sup>, however, fewer data exist after vaporized<sup>[13]</sup> and edible THC.<sup>[17,21]</sup> As inhalation via vaporization and oral cannabis are becoming increasingly popular routes of intake,<sup>[14]</sup> controlled administration studies examining these routes are crucial to understanding cannabinoid OF pharmacokinetics. Further, ideally these routes are studied in a within-subject, placebo-controlled design to best compare cannabinoid pharmacokinetics.

THC is metabolized to the active metabolite 11-hydroxy-THC (11-OH-THC), and to the inactive metabolite 11-nor-9-carboxy-THC (THCCOOH). Concentrations for THC alone<sup>[17]</sup> and with

THCCOOH<sup>[21]</sup> were recently described in OF following an edible cannabis brownie administration. High THC OF concentrations primarily result from oral mucosa contamination during smoking or vaporization, with minor contribution from THC that partitions from blood into OF, especially during and shortly after intake. OF THCCOOH concentrations vary considerably between occasional and frequent cannabis smokers.<sup>[13,15,16]</sup> Minor cannabinoids present in the cannabis plant include cannabinol (CBN), cannabidiol (CBD), cannabigerol (CBG), and  $\Delta^9$ -tetrahydrocannabivarin (THCV). These minor cannabinoids are possible markers of recent cannabis intake; however, limited CBN and CBD OF concentration profiles are available after controlled smoked<sup>[15]</sup> and vaporized<sup>[13]</sup> cannabis administration. There are no CBN or CBD OF data after oral cannabis

\* Correspondence to: Karl B. Scheidweiler, Chemistry and Drug Metabolism, Clinical Pharmacology and Therapeutics Research Branch, NIDA-IRP, NIH Biomedical Research Center, 251 Bayview Blvd, Baltimore, MD 21224, USA. E-mail: kscheidweiler@intra.nida.nih.gov

a Chemistry and Drug Metabolism Section, Clinical Pharmacology and Therapeutics Branch, National Institute on Drug Abuse Intramural Research Program, National Institutes of Health, Baltimore, MD, USA

b Program in Toxicology, University of Maryland Baltimore, Baltimore, MD, USA

c University of Maryland School of Medicine, Baltimore, MD, USA

administration. Additionally, to our knowledge, OF CBG and THCV disposition were not yet investigated.

With the increase in OF drug testing and increasing knowledge of OF drug disposition, cut-offs and testing criteria need to be established for clinical and forensic drug-testing programmes. For DUID, the European Driving under the Influence of Drugs, Alcohol, and Medicines (DRUID) project implemented a THC  $\geq 1 \mu\text{g/L}$  analytical cut-off in OF.<sup>[22]</sup> For workplace testing, the Substance Abuse and Mental Health Services Administration (SAMHSA) proposed a THC  $\geq 2 \mu\text{g/L}$  confirmatory cut-off in OF.<sup>[23]</sup> However, these cut-offs need to be fully evaluated following controlled cannabis administration via routes other than inhalation via smoking (i.e., inhalation via vaporization or oral).

In order to fully characterize cannabinoid disposition in OF, we investigated THC, metabolites, and minor cannabinoids in OF (quantifying THC, 11-OH-THC, THCCOOH, THCV, CBD, and CBG) following controlled smoked, vaporized, and oral brownie cannabis administration in frequent and occasional cannabis smokers. Quantification of a wide spectrum of OF cannabinoids also permits assessment of detection windows for parent cannabinoids and metabolites improving interpretation of cannabinoid OF results.

## Materials and methods

### Participants

Healthy cannabis users (18–50 years) were recruited for this study which has been approved by the National Institute on Drug Abuse (NIDA) Intramural Research Program Institutional Review Board, the Food and Drug Administration (FDA), and the Drug Enforcement Administration (DEA). Individuals were recruited by radio and printed advertisements and participant referrals. All participants underwent a comprehensive medical and psychological evaluation. Inclusion criteria were self-reported cannabis intake  $\geq 2\times$  per month but  $<3\times$  per week (occasional smokers) or  $\geq 5\times$  per week (frequent smokers) over the previous three months, and frequent smokers had to produce a positive urine cannabinoid screen. Exclusion criteria included blood pressure  $>140/90$  mmHg or heart rate  $>100$  bpm at rest; clinically significant electrocardiogram abnormality; inability to discontinue contraindicated medication before study dosing; physical dependence on any drug other than cannabis, caffeine, or nicotine; medicinal cannabis use; history of clinically significant medical or neurological illness or adverse event associated with cannabis intoxication; recent blood donation  $>450$  mL; pregnant or nursing women; recent interest or participation in a drug abuse treatment programme; and any history of food allergy or sensitivity to gluten, dairy, egg, soy, and/or chocolate. Pregnancy tests were administered at screening and on each session admission to women with reproductive potential. Individuals provided written, informed consent before admittance to the study.

### Study design

The study was randomized, double-blind, and placebo-controlled with a crossover and double-dummy design. Participants entered the secure research unit  $\sim 19$  h before dosing to preclude acute intoxication. Cannabis cigarettes were obtained through the NIDA Drug Supply Program. Active cigarettes ( $0.734 \pm 0.05$  g) contained  $6.9 \pm 0.95\%$  ( $\sim 50.6$  mg) THC and  $0.20 \pm .01\%$  ( $\sim 1.5$  mg) CBD. Placebo cigarettes ( $0.713 \pm 0.05$  g) contained  $0.001 \pm 0.000\%$  THC and no detectable CBD. Throughout four dosing sessions, participants were administered one active or placebo brownie followed by one active

or placebo cigarette or one active or placebo vaporized ground cannabis dose ( $210^\circ\text{C}$ , Volcano® Medic, Storz & Bickel, Tuttlingen, Germany). No more than one active dose was administered per session and the oral dose was followed by either smoking or vaporization in two sessions each. Participants had 10 min to consume the oral dose *ad libitum* followed by 10 min to consume the inhaled dose *ad libitum*. Frequent smokers remained on the unit 72 h post-dose and were required to leave the unit for  $\geq 72$  h before being admitted to their next session. Occasional smokers remained on the unit 54 h post-dose but had the option of remaining on the unit for multiple sessions; they were not dosed more frequently than their self-reported intake frequency.

Brownies were prepared with Duncan Hines® double fudge brownie mix according to the manufacturer's instructions and wet batter was portioned into a muffin tray. The contents of either an active or placebo cigarette were ground, placed into a greased foil packet, and baked at  $121^\circ\text{C}$  for 30 min to ensure decarboxylation of the acid precursor to THC, then mixed into an individual portion of brownie batter. After cooling, brownies were stored at  $-20^\circ\text{C}$  until the night before dosing, and thawed at  $4^\circ\text{C}$ .

OF specimens were collected with the Quantisal™ device (Immunoanalysis, Pomona, CA, USA), which has a volume adequacy indicator for  $1.0 \pm 0.1$  mL OF. OF was collected until the indicator turned blue or 5 min elapsed, whichever occurred first due to the tight timeline. Oral intake was prohibited 10 min prior to OF collection. OF was collected on admission ( $-19$  h), 1.5 h before the initiation of smoking/vaporization (baseline,  $-1.5$  h) and at 0.17, 1.5, 3.5, 5, 8, 10, 12, 14, 20, 26, 32, 38, 44, and 50 h after smoking/vaporization initiation, and at 54 h for occasional smokers only, and at 56, 62, 68, and 72 h for frequent smokers only.

### Oral fluid analysis

Specimens were placed in 3 mL elution/stabilizing buffer at  $4^\circ\text{C}$  for  $>12$  h prior to pad removal, followed by transfer of OF/buffer to polypropylene cryotubes and storage at  $4^\circ\text{C}$  until analysis. Specimens were analyzed within 1 month of collection based on our previous OF cannabinoid stability study<sup>[24,25]</sup> and quantified for THC, THCCOOH, 11-OH-THC, THCV, CBD, and CBG by a previously published method.<sup>[26]</sup> Briefly, samples (1 mL elution buffer OF mixture containing 0.25 mL OF) were mixed with 0.3 mL 1 M ammonium acetate buffer (pH 4) and hydrolyzed with 625 Units of  $\beta$ -glucuronidase (BG100®, Kura Biotech, Puerto-Varas, Chile), acidified and extracted with cation exchange solid-phase columns. Cannabinoids were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using atmospheric pressure chemical ionization with  $0.2 \mu\text{g/L}$  limits of quantification (except  $15 \text{ ng/L}$  THCCOOH). Inter-assay accuracy and imprecision were 88.1–106% and 5.8–8.2%CV, respectively ( $n = 92$ ). Samples quantifying greater than the upper limits of quantification were re-analyzed after dilution with OF/buffer.

### Data analysis

Differences in demographic data between groups were evaluated with t-tests. Maximum concentration ( $C_{\text{max}}$ ), time to  $C_{\text{max}}$  ( $t_{\text{max}}$ ), and time of last detection ( $t_{\text{last}}$ ) were calculated with concentrations observed post-dose and differences between administration routes were assessed with SPSS® Statistics 23 for Windows (IMB, Armonk, NY, USA). For analytes detected after all three routes, differences were evaluated by repeated-measures ANOVA with separation of frequent and occasional smokers. If sphericity was violated, the

Greenhouse-Geisser correction was utilized. If a significant route effect was observed, planned Helmert contrasts were performed first by comparing mean oral dose to the combined mean of inhaled doses then comparing smoking to vaporization. Smoking group differences (frequent vs. occasional) were evaluated with separate repeated-measures ANOVA, with group included as a between-subject factor; if a significant route\*group interaction was observed, then group differences after each administration route were evaluated with *t*-tests. Significance was attributed to a two-tailed  $p < 0.05$ .

## Results

### Participants

Demographics for 11 frequent and 9 occasional cannabis smokers are summarized in Table 1. Participants were 19–46 years old, 75% male and 75% African American. Participant K was originally admitted as an occasional smoker, but later reclassified as a frequent smoker based on baseline and post-dose blood cannabinoid

pharmacokinetics. Participant H smoking frequency at admission to Session 1 was inconsistent with self-reported frequency at screening so his demographic data were not included in summary statistics; smoking frequencies reported on admission to subsequent sessions were consistent with self-reported frequency at screening. Frequent smokers were all African American, began smoking at a significantly younger age, smoked significantly more frequently over the previous 14 days, and smoked significantly more per smoking occasion.

In total, 1102 OF specimens (598 frequent, 504 occasional) were analyzed. OF specimens were not collected from 56 to 72 h for participant K because he was originally recruited as an occasional smoker.

### Pharmacokinetic evaluation

Pharmacokinetic parameters and statistical evaluations for cannabinoids and metabolites are summarized in Table 2 for analytes detected after all three routes. Time course profiles for all six analytes in frequent and occasional smokers following three

**Table 1.** Demographic data and cannabis smoking histories for 11 frequent and 9 occasional smokers.

Participant	Sex	Age (years)	Race <sup>a</sup>	BMI (kg/m <sup>2</sup> )	Age at first use <sup>b</sup> (years)	Lifetime Years Smoked <sup>b</sup>	Cannabis Intake Frequency <sup>b</sup>	Time between last use and admission <sup>c</sup>	Number of days used in last 14 <sup>c</sup>	Average joint or joint equivalent per smoking occasion <sup>c</sup>
<b>Frequent Smokers</b>										
A	M	21	AA	26.5	16	5	Daily	17.2 h	14	5
B	M	22	AA, U	31.0	15	7	Daily	19.3 h	10	4
C	M	19	AA	19.8	13	6	Daily	18.7 h	14	4
D	F	23	AA	31.9	13	10	Daily	7.9 h	14	10
E	M	38	AA	32.2	12	26	Daily	2.4 h	14	15
F	F	29	AA	31.0	11	18	Daily	1.9 h	14	20
G	M	38	AA	22.0	16	22	Daily	2.1 h	14	7
H	M	34	AA	23.0	14	20	5x/week	239.7 h <sup>d</sup>	2 <sup>d</sup>	2 <sup>d</sup>
I	M	21	AA	25.0	11	10	Daily	0.7 h	14	5
J	M	25	AA	19.0	13	12	5x/week	5.8 h	14	2
K	M	31	AA	16.8	15	16	2-3x/week <sup>e</sup>	5.1 h <sup>e</sup>	4 <sup>e</sup>	2.5 <sup>e</sup>
Mean		27.4 <sup>f</sup>		25.3	13.5 <sup>f</sup>	13.9		8.4 h	13.6 <sup>f</sup>	8.0 <sup>f</sup>
SD		6.9		5.6	1.8	6.9		7.8 h	1.3	6.0
Median		25.3		25.0	13.0	12.3		5.8 h	14.0	5.0
<b>Occasional Smokers</b>										
L	M	24	AA	36.3	17	7	2x/month	1.4 days	3	2
M	M	21	AA	23.0	13	8	2x/week	0.7 days	4	2
N	M	25	W	24.2	21	4	2x/week	13.0 days	1	3
O	M	40	W	28.3	18	22	2x/week	30.7 days	0	2
P	F	46	AA	31.0	26	20	2x/week	0.4 days	4	4
Q	M	33	AA	30.7	16	17	2x/month	22.8 days	0	3
R	F	22	W	22.0	16	6	2x/week	1.7 days	4	1
S	F	22	W	23.0	14	8	2x/week	1.1 days	10	2
T	M	31	W	21.7	22	9	1-2x/week	1.8 days	2	2
Mean		29.4 <sup>f</sup>		26.7	18.1 <sup>f</sup>	11.3		8.2 days	3.1 <sup>f</sup>	2.3 <sup>f</sup>
SD		8.6		5.1	4.2	6.3		11.4 days	3.1	0.9
Median		24.9		24.2	17.0	8.5		1.7 days	3.0	2.0

<sup>a</sup>AA, African American; W, white; U, unknown

<sup>b</sup>Data collected during screening

<sup>c</sup>Data collected on admission to Session 1

<sup>d</sup>Self-reported data on admission inconsistent with data received at screening. Data excluded from statistics.

<sup>e</sup>Self-reported data inconsistent with biological sample concentrations. Data excluded from statistics.

<sup>f</sup>Significant difference between groups ( $p < 0.05$ )

**Table 2.** Summary of mean (range), maximum cannabinoid analyte concentrations ( $C_{max}$ ), time to  $C_{max}$  ( $t_{max}$ ), and time of last positive ( $t_{last}$ ) after smoked, vaporized and oral cannabis doses (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC; ~50.6 mg THC) in 11 frequent and 9 occasional smokers. Repeated-measures analysis of variance F-statistic and p-value for overall route effect and planned Helmert contrasts are reported (contrast 1 evaluated the difference between the variance from oral dosing and the combined variance from smoked and vaporized dosings, contrast 2 evaluated the difference in variances from smoked and vaporized dosing). Route-specific significant differences between smoking groups were calculated and annotated in table. Only participants whose oral fluid was positive for analytes after all cannabis administrations were included in analyses.

Analyte and Parameter	N	Oral	Smoking	Vaporization	F	p	Contrast 1		Contrast 2	
							F	p	F	p
THC										
Frequent Smokers										
C <sub>max</sub> µg/L	11	297 (16.5-938)	2789 (141-8503)	1874 (68.6-7373)	3.60	<b>0.046</b>	11.63	<b>0.007</b>	0.648	0.440
t <sub>max</sub> h	11	0.41 (0.17-1.5)	0.17	0.17	2.22	0.167	-	-	-	-
t <sub>last</sub> h <sup>a</sup>	10	55.0 (20- >72)	61.0 (32- >72)	55.4 (26- >72)	0.80	0.463	-	-	-	-
Occasional Smokers										
C <sub>max</sub> µg/L	9	202 (65-380)	837 (81.4-5914)	545 (7.6-3279)	1.00	0.388	-	-	-	-
t <sub>max</sub> h	9	0.32 (0.17-1.5)	0.17	0.32 (0.17-1.5)	0.47	0.633	-	-	-	-
t <sub>last</sub> h	9	24.7 (10- >54)	24.7 (8-50)	18.6 (5- >54)	2.37	0.126	-	-	-	-
11-OH-THC										
Frequent Smokers										
C <sub>max</sub> µg/L	1	0.23	2.2	0.27	-	-	-	-	-	-
t <sub>max</sub> h	1	0.17	0.17	0.17	-	-	-	-	-	-
t <sub>last</sub> h	1	0.17	0.17	0.17	-	-	-	-	-	-
Occasional Smokers										
C <sub>max</sub> µg/L	0	-	-	-	-	-	-	-	-	-
t <sub>max</sub> h	0	-	-	-	-	-	-	-	-	-
t <sub>last</sub> h	0	-	-	-	-	-	-	-	-	-
THCCOOH <sup>s</sup>										
Frequent Smokers										
C <sub>max</sub> µg/L	11	0.40 (0.13-1.2)	0.41 (0.13-1.2)	0.37 (0.05-0.97)	0.09	0.911	-	-	-	-
t <sub>max</sub> h	11	25.7 (0.17-68)	14.2 (0.17-68)	8.4 (0.17-20)	4.29	<b>0.028</b>	7.41	<b>0.021</b>	0.96	0.351
t <sub>last</sub> h <sup>a</sup>	10	71.6 (68- >72)	66.6 (44- >72)	66.6 (44- >72)	1.74	0.204	-	-	-	-
Occasional Smokers										
C <sub>max</sub> µg/L	3	0.35 (0.02-0.80)	0.18 (0.12-0.31)	0.09 (0.02-0.17)	0.96	0.457	-	-	-	-
t <sub>max</sub> h	3	8.5 (3.5-14)	0.17	6.8 (0.17-20)	0.84	0.498	-	-	-	-
t <sub>last</sub> h	3	38.7 (8- >54)	15.7 (1.5-44)	18.6 (0.17- >54)	1.89	0.265	-	-	-	-
THCV										
Frequent Smokers										
C <sub>max</sub> µg/L	11	4.5 (0.26-14.5)	28.3 (1.6-82.4)	40.2 (1.8-159)	2.74	0.117	-	-	-	-
t <sub>max</sub> h	11	0.53 (0.17-1.5)	0.29 (0.17-1.5)	0.17	1.89	0.193	-	-	-	-
t <sub>last</sub> h	11	1.7 (0.17-3.5)	4.7 (1.5-12)*	3.9 (1.5-8)	5.25	<b>0.015</b>	17.78	<b>0.002</b>	0.47	0.510
Occasional Smokers										
C <sub>max</sub> µg/L	7	3.5 (1.2-5.6)	34.3 (1.0-146)	17.5 (0.30-82.4)	1.63	0.237	-	-	-	-
t <sub>max</sub> h	7	0.36 (0.17-1.5)	0.17	0.17	1.00	0.356	-	-	-	-
t <sub>last</sub> h	7	2.7 (0.17-5)	1.7 (0.17-8)*	2.8 (0.17-8)	0.647	0.541	-	-	-	-
CBD										
Frequent Smokers										
C <sub>max</sub> µg/L	11	8.0 (0.48-26.3)	93.3 (0.65-350)	76.3 (2.3-339)	2.92	0.077	-	-	-	-
t <sub>max</sub> h	11	0.53 (0.17-1.5)	0.29 (0.17-1.5)	0.29 (0.17-1.5)	1.00	0.386	-	-	-	-
t <sub>last</sub> h	11	2.2 (1.5-3.5)	8.1 (1.5-20)	7.4 (3.5-20)	5.40	<b>0.013</b>	21.55	<b>0.001</b>	0.09	0.769
Occasional Smokers										
C <sub>max</sub> µg/L	9	5.9 (2.1-11.4)	55.9 (2.5-291)	28.2 (0.23-167)	1.731	0.209	-	-	-	-
t <sub>max</sub> h	9	0.47 (0.17-1.5)	0.17	0.17	2.29	0.169	-	-	-	-
t <sub>last</sub> h	9	2.9 (1.5-5)	4.0 (0.17-20)	3.6 (0.17-20)	0.19	0.728	-	-	-	-
CBG										
Frequent Smokers										
C <sub>max</sub> µg/L	11	17.0 (0.77-60.6)	165 (5.7-441)	87.4 (2.7-394)	4.63	<b>0.022</b>	10.56	<b>0.009</b>	1.87	0.201
t <sub>max</sub> h	11	0.41 (0.17-1.5)	0.17	0.17	2.22	0.167	-	-	-	-
t <sub>last</sub> h	11	3.0 (1.5-8)	10.6 (5-20)	5.4 (1.5-8)	8.64	<b>0.002</b>	15.52	<b>0.003</b>	5.64	<b>0.039</b>

(Continues)



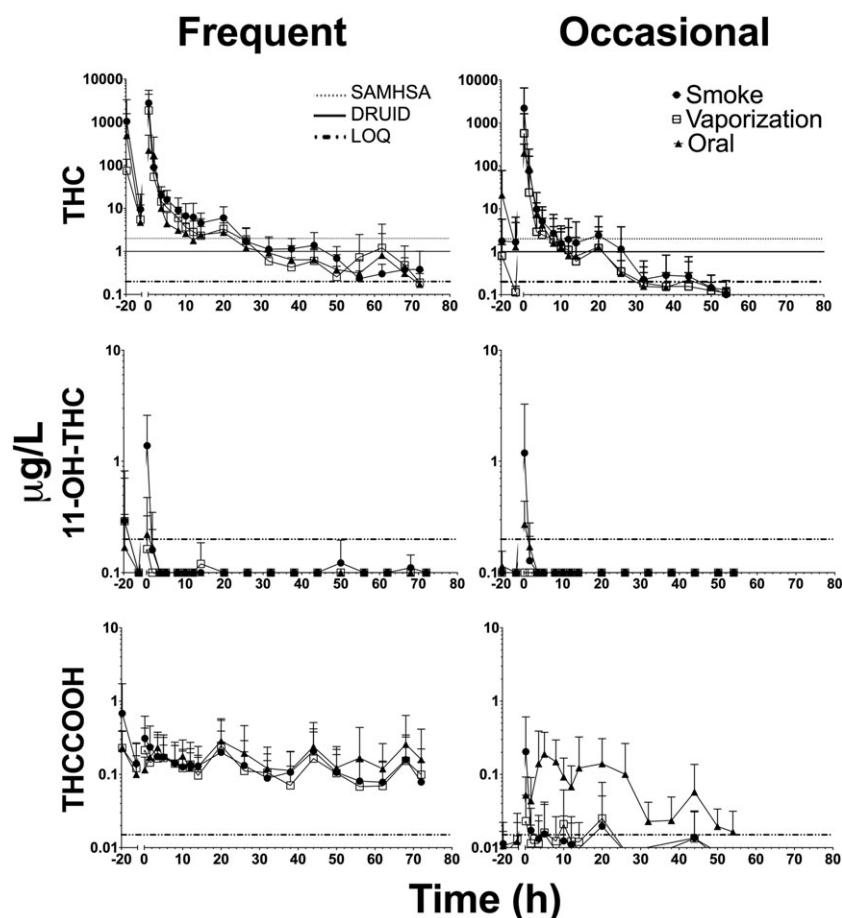
**Table 2.** (Continued)

Analyte and Parameter	N	Oral	Smoking	Vaporization	F	p	Contrast 1		Contrast 2	
							F	p	F	p
Occasional Smokers										
C <sub>max</sub> , µg/L	9	11.9 (3.2-26.3)	118 (5.4-602)	24.4 (0.30-142)	2.19	0.176	-	-	-	-
t <sub>max</sub> , h	9	0.47 (0.17-1.5)	0.17	0.17	2.29	0.169	-	-	-	-
t <sub>last</sub> , h	9	3.7 (1.5-10)	4.9 (0.17-26)	3.2 (0.17-20)	0.63	0.465	-	-	-	-
THC, Δ <sup>9</sup> -tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; THCV, Δ <sup>9</sup> -tetrahydrocannabivarin; CBD, cannabidiol; CBG, cannabigerol; bolded p-values designate significance										
*denotes route-specific significant difference between smoking groups (two-tailed t-test p < 0.05)										
<sup>a</sup> N = 10 because participant K (originally recruited as an occasional smoker, therefore oral fluid was only collected up to 54 h post-dose) was still positive at the final collection.										
<sup>s</sup> total THCCOOH (free + hydrolyzed glucuronide)										

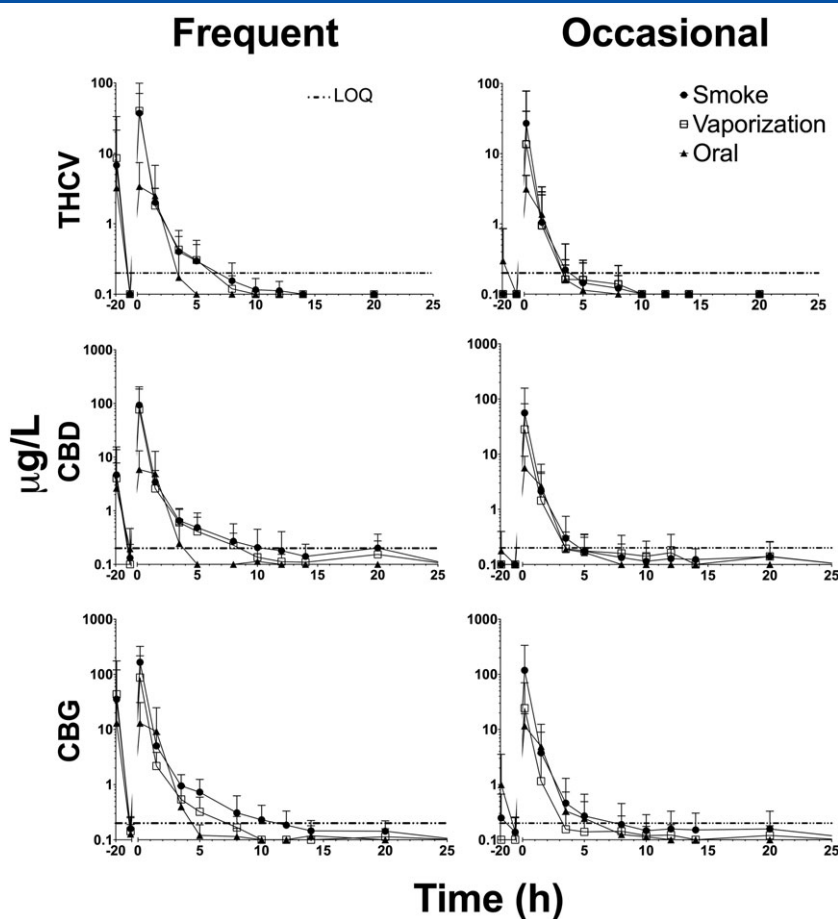
administration routes are shown in Figures 1 and 2. THCCOOH concentrations represent free and hydrolyzed glucuronide concentrations. THC-glucuronide present in OF would also be hydrolyzed by this method (67% efficiency)<sup>[26]</sup> but biological concentrations are considered negligible based on previous research.<sup>[27]</sup>

Observed mean THC, 11-OH-THC, THCV, CBD, and CBG  $t_{\max}$  occurred at or before the first OF collection (0.17 h) immediately at the completion of cannabis intake, followed by rapid

concentration decreases for frequent and occasional smokers after all routes of administration. There were no significant differences in THC, 11-OH-THC, THCV, CBD, and CBG  $t_{\max}$  between routes. Frequent smokers' THCCOOH mean (range)  $t_{\max}$  was significantly later after oral dosing (25.7 [0.17–68] h) compared to smoking (14.2 [0.17–68] h) and vaporization (8.4 [0.17–20] h), as described in Table 2, Contrast 1. Only three occasional smokers were THCCOOH positive after all routes of administration; however, all occasional



**Figure 1.** Mean + standard deviation (SD) concentrations on a log-scale for  $\Delta^9$ -tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) in  $n = 11$  frequent (left) and  $n = 9$  occasional (right) smokers up to 72 and 54 h, respectively, after smoked, vaporized, and oral cannabis (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC; ~50.6 mg THC) administration (0 h). Horizontal lines present at the limits of quantification (LOQ; 0.2  $\mu\text{g/L}$  for all, except 15 ng/L for THCCOOH) and OF THC cut-offs for DRUID (1  $\mu\text{g/L}$ ) and SAMHSA (2  $\mu\text{g/L}$ ).



**Figure 2.** Mean + standard deviation (SD) concentrations (up to 20 h) for  $\Delta^9$ -tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabigerol (CBG) in  $n = 11$  frequent (left) and  $n = 9$  occasional (right) smokers after smoked, vaporized, and oral cannabis (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC; ~50.6 mg THC) administration (0 h). Horizontal lines present at the limits of quantification (0.2  $\mu\text{g/L}$  for all)

smokers were positive after the oral dose. 11-OH-THC was only detected in one frequent smoker after all doses; however, 11-OH-THC was present in 10/11, 2/11, and 4/11 frequent smokers after the smoked, vaporized, and oral doses, respectively. 11-OH-THC was detected in 3/9, 0/9, and 6/9 occasional smokers after smoked, vaporized, and oral cannabis administration. CBD and CBG were detected in all participants after all administrations, while THCV was detected in all frequent smokers and 9/9, 7/9, and 9/9 occasional smokers after smoked, vaporized, and oral cannabis.

Frequent smokers' mean THC and CBG  $C_{\text{max}}$  were significantly greater after inhaled routes than after oral cannabis; no difference was observed between smoked and vaporized cannabis. Mean (range) THC  $C_{\text{max}}$  in frequent smokers after smoked (2789 [141–8503]  $\mu\text{g/L}$ ) and vaporized (1874 [68.6–7373]  $\mu\text{g/L}$ ) cannabis were significantly higher than in occasional smokers after smoked (837 [81.4–5914]  $\mu\text{g/L}$ ) and vaporized (545 [7.6–3279]  $\mu\text{g/L}$ ) cannabis. Mean (range) THC  $C_{\text{max}}$  after oral administration for frequent and occasional smokers were 297 (16.5–938) and 202 (65.0–380)  $\mu\text{g/L}$ , respectively. Overall, frequent smokers' observed THC  $C_{\text{max}}$  were significantly greater than those in occasional smokers', regardless of route; no statistically significant route\*group interactions were observed.

Frequent smokers' mean (range) THCV, CBD, and CBG  $t_{\text{last}}$  were significantly later after smoked (4.7 [1.5–12], 8.1 [1.5–20], and 10.6 [5–20] h, respectively) and vaporized (3.9 [1.5–8], 7.4 [3.5–20], and 5.4 [1.5–8] h, respectively) cannabis compared to oral (1.7

[0.17–3.5], 2.2 [1.5–3.5], and 3 [1.5–8] h, respectively) administration. Minor cannabinoids THCV, CBD, and CBG were never detected beyond 26 h in any participant after any administration route; CBD (up to 20 h) and CBG (up to 26 h) were detected longer than THCV (up to 12 h). For frequent smokers, the only significant difference between inhaled routes was a later CBG  $t_{\text{last}}$  after smoked cannabis compared to the vaporized dose. Cannabinoid  $t_{\text{last}}$  in occasional smokers were not significantly different between smoked and vaporized administration. When comparing groups, a significantly later THCV  $t_{\text{last}}$  was observed for frequent smokers after smoking compared to occasional smokers.

At their final collection time (72 h), 6/11, 3/11, and 2/11 frequent smokers were still THC positive at 0.2–2.2, 0.2–0.6, 0.3–0.5  $\mu\text{g/L}$  after smoked, vaporized, and oral cannabis, respectively. Only one occasional smoker was THC positive at the final collection time (54 h) after the vaporized and oral sessions, with 0.2 and 0.4  $\mu\text{g/L}$ , respectively; this participant had the largest  $C_{\text{max}}$  in these sessions among occasional smokers. Overall, frequent smokers' THC  $t_{\text{last}}$  was significantly later than occasional smokers', regardless of route; no statistically significant route\*group interactions were observed. THCCOOH was present at discharge in 8/11, 7/11, and 10/11 frequent smokers and 0/9, 1/9, and 2/9 occasional smokers after smoked, vaporized, and oral cannabis, respectively. Occasional participants P and S with THCCOOH concentrations > LOQ (limit of quantification) at discharge were also THCCOOH positive at admission (–19 h) and baseline (–1.5 h) to the same session, with



THCCOOH concentrations at admission comparable to those at discharge. THCCOOH  $t_{\text{last}}$  was significantly later in frequent smokers compared to occasional smokers, regardless of administration route. 11-OH-THC was detected infrequently and never beyond 1.5 h in any participant after any administration route.

### Cannabinoid detection rates

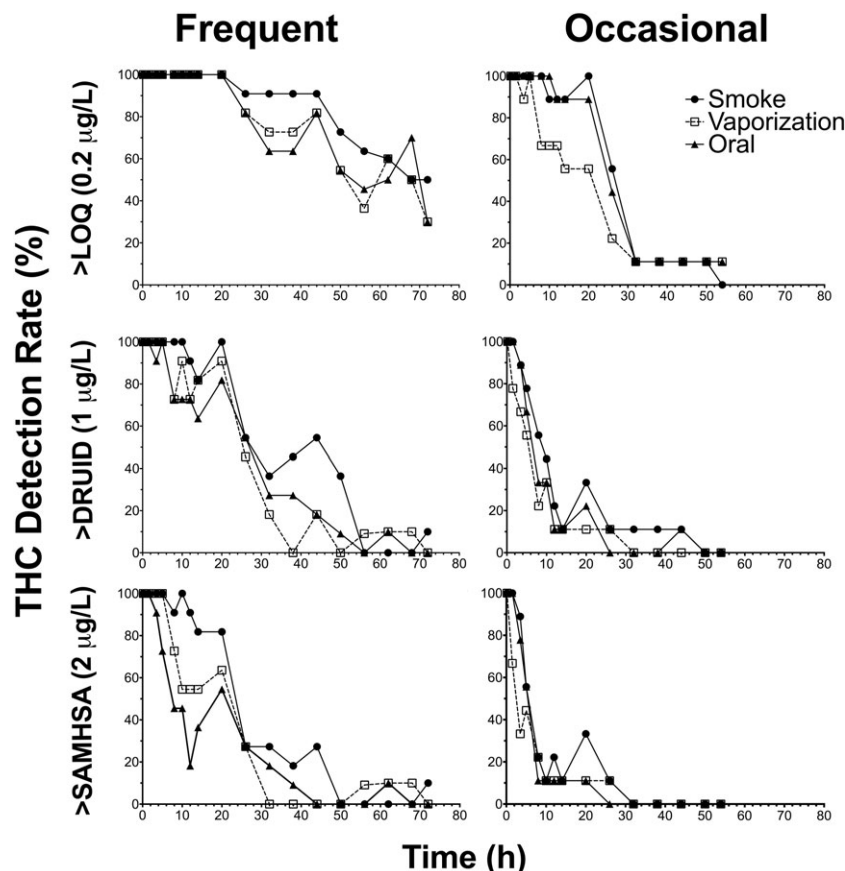
THC detection rates at three cut-offs (LOQ 0.2  $\mu\text{g/L}$ , DRUID 1  $\mu\text{g/L}$ , and SAMHSA 2  $\mu\text{g/L}$ ) for frequent and occasional smokers are found in Figure 3. At the LOQ, DRUID, and SAMHSA cut-offs, THC was still observed in frequent smokers' OF samples at discharge (72 h), with detection rates never reaching 0%. At the LOQ, one occasional smoker's OF samples were still THC positive at discharge (54 h), while all samples were below DRUID and SAMHSA THC cut-offs by 50 and 32 h, respectively. Detection rates dropped more quickly following oral administration. One frequent smoker was THC positive (2.2  $\mu\text{g/L}$ ) above DRUID and SAMHSA cut-offs when discharged 72 h after smoking. At baseline (-1.5 h), following an overnight stay on the controlled research unit, 11/11, 9/11, and 10/11 frequent smokers remained positive for THC above DRUID cut-off (1  $\mu\text{g/L}$ ) prior to smoked, vaporized, and oral cannabis sessions, respectively. Of the frequent smokers THC positive at baseline, all except four samples' THC concentrations were also above the SAMHSA THC cut-off (2  $\mu\text{g/L}$ ) prior to a dosing session. At baseline, only one occasional smoker was THC positive above the SAMHSA cut-off prior to smoking (14.3  $\mu\text{g/L}$ ) and oral (10.6  $\mu\text{g/L}$ ) sessions.

THCV, CBD, and CBG detection rates at the LOQ (0.2  $\mu\text{g/L}$ ) for frequent and occasional smokers are found in Figure 4. More frequent than occasional smokers were positive for the minor cannabinoids after all routes, since THCV was not detected after vaporization in some occasional smokers. At the LOQ, frequent smokers were no longer positive for THCV, CBD, and CBG at 14, 26, and 26 h, respectively, and 10, 26, and 32 h for occasional smokers. Frequent smokers' detection rates were highest for the longest amount of time after smoked cannabis administration, as smoking produced slightly higher cannabinoid concentrations compared to vaporized and oral doses. Detection rates between frequent and occasional smokers were similar for minor cannabinoids following vaporized and oral administration.

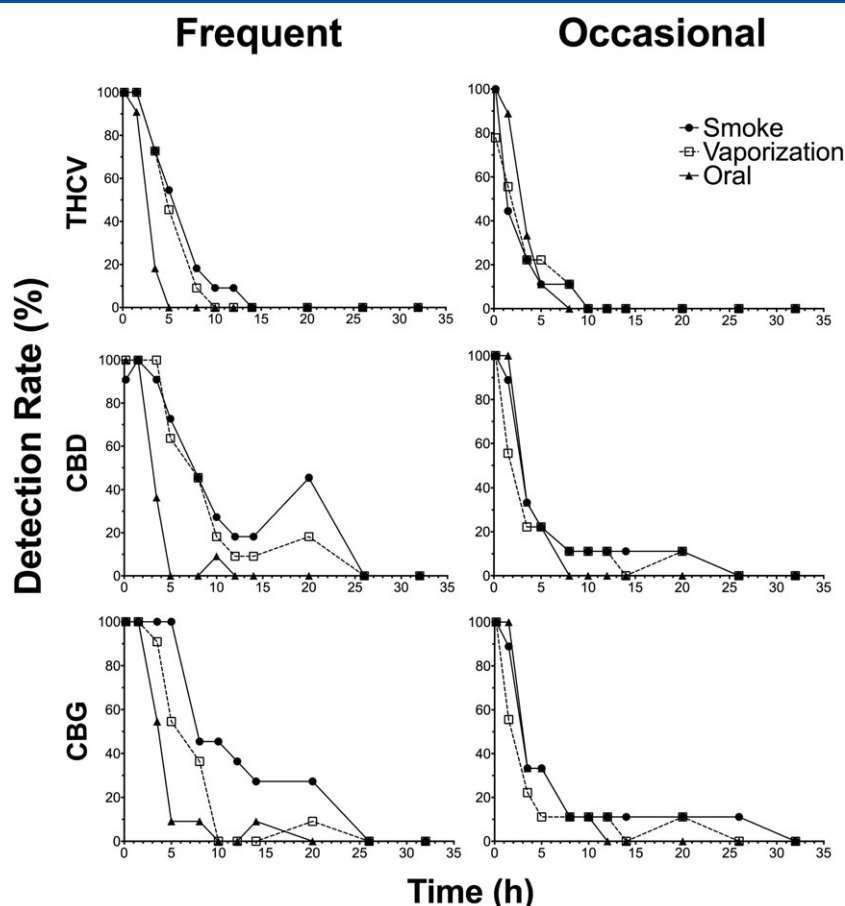
In order to establish detection windows reflecting use within the last 24 h, different cut-offs were investigated for the minor cannabinoids. THCV, CBD, and CBG detection rates at proposed 0.3, 0.5, and 0.3  $\mu\text{g/L}$  cut-offs, respectively, for frequent and occasional smokers are found in Figure 5. At these cut-offs, THCV, CBD, and CBG were no longer detected in frequent smokers OF samples at 10, 26, and 26 h, respectively, and 10, 14, and 26 h for occasional smokers. Detection rates between frequent and occasional smokers were similar for minor cannabinoids at 0.3–0.5  $\mu\text{g/L}$  cut-offs.

### Discussion

For the first time, we present parent cannabinoid (THC, THCV, CBD, and CBG) and metabolite (11-OH-THC and THCCOOH) disposition



**Figure 3.**  $\Delta^9$ -tetrahydrocannabinol (THC) detection rates (%) at three cut-offs: limit of quantification (0.2  $\mu\text{g/L}$ , LOQ), DRUID (1  $\mu\text{g/L}$ ), and SAMHSA (2  $\mu\text{g/L}$ ) for  $n=11$  frequent (left) and  $n=9$  occasional (right) smokers up to 72 and 54 h, respectively, after smoked, vaporized, and oral cannabis (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC; ~50.6 mg THC) administration (0 h).



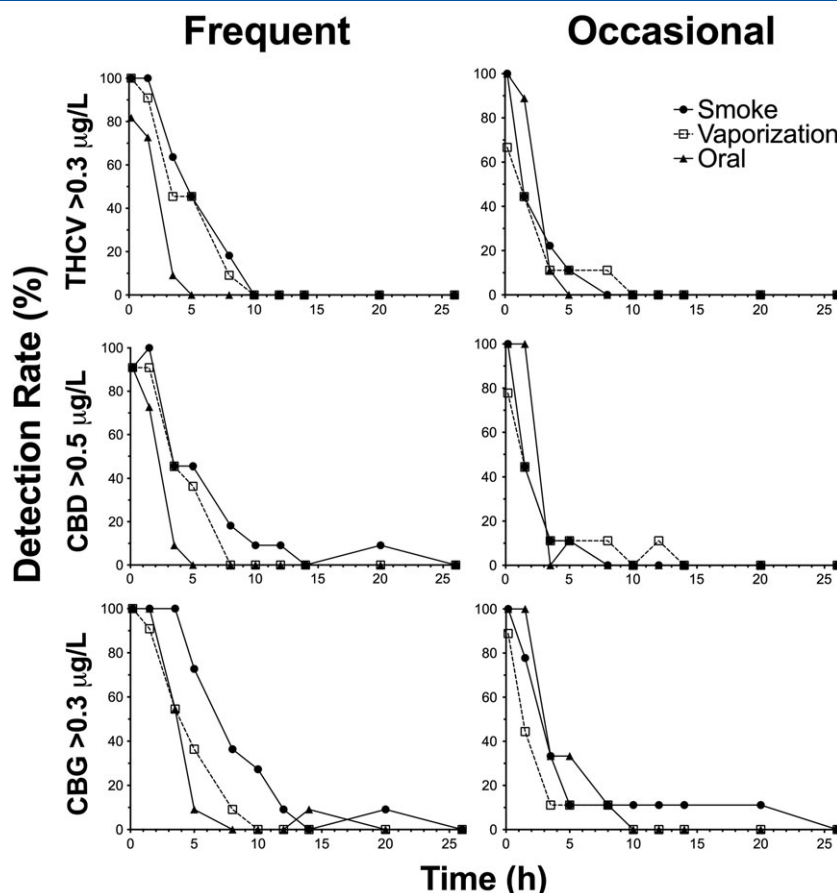
**Figure 4.**  $\Delta^9$ -tetrahydrocannabivarin (THCV), cannabidiol (CBD), and cannabigerol (CBG) detection rates (%) up to 32 h at the limits of quantification ( $0.2 \mu\text{g/L}$ ) for  $n = 11$  frequent (left) and  $n = 9$  occasional (right) smokers after smoked, vaporized, and oral cannabis (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC;  $\sim 50.6 \text{ mg}$  THC) administration (0 h)

in OF following controlled smoked, vaporized, and oral (brownie) cannabis administration utilizing a within-subject study design for 11 frequent and 9 occasional cannabis smokers. Cannabinoid pharmacokinetics are well studied in OF following smoked<sup>[9,15,16]</sup> administration of cannabis, while clinical data following vaporized<sup>[13]</sup> and edible<sup>[17,21]</sup> cannabis are limited.

THC, THCV, CBD, and CBG  $t_{\text{max}}$  indicate oral mucosa contamination from cannabis intake that is observed at the first OF collection time point. 11-OH-THC also appeared immediately (0.17 h) in a few cases suggesting possible THC metabolism in the oral mucosa. Cytochrome P450 enzymes were identified in human oral tissue cells<sup>[28–31]</sup> and could contribute to the presence of metabolites in OF. 11-OH-THC was rarely detected after all administration routes and never beyond 1.5 h post-dose. 11-OH-THC was detected more frequently after smoking in this study compared to previous studies due to our lower LOQ (0.2 vs.  $0.5 \mu\text{g/L}$ ). Most observed 11-OH-THC concentrations would have been missed with previous analytical methods. THCCOOH appeared immediately (0.17 h) in a few occasional smokers' OF after inhaled routes but more frequently and for longer periods of time after oral intake. Among occasional smokers THCCOOH positive at 0.17 h (4/9, 3/9, and 7/9 after smoked, vaporized, and oral doses, respectively), concentrations were greater than those at baseline in all but one case, suggesting THC metabolism in the oral mucosa and/or partitioning from blood. THCCOOH concentrations remained elevated in frequent smokers' OF throughout the sessions, although the  $t_{\text{max}}$  also was delayed after oral administration, similar to the pattern observed in occasional

smokers. A significantly later THCCOOH  $t_{\text{max}}$  also occurred in blood specimens from the same cohort following oral cannabis administration, supporting blood-OF partitioning. Similarly, Vandrey *et al.* observed delayed mean (range) OF THCCOOH  $t_{\text{max}}$  of 9.8 (3–30) and 17.4 (0–54) h following consumption of 25 and 50 mg oral THC (brownie), respectively.<sup>[21]</sup>

Frequent smokers' THC  $C_{\text{max}}$  after smoking a 6.9% THC cannabis cigarette in the present study were higher than those reported previously for similar potency cannabis,<sup>[15,16]</sup> but our initial OF collection time post-dose (0.17 vs. 0.5 h) was earlier. However, Toennes *et al.* collected OF 0.08 h after smoking a 500  $\mu\text{g}$  THC/kg cannabis cigarette and observed higher median (range) THC  $C_{\text{max}}$  of 6202 (387–71 147) and 1242 (397–6438) ng/g in frequent and occasional smokers, respectively.<sup>[9]</sup> Occasional smokers' median THC  $C_{\text{max}}$  in the present study were lower than those previously reported<sup>[15,16]</sup> but exhibited a wider range, which could be influenced by smoking history, topography, and possible titration. We observed lower median (range) THC concentrations for both groups of smokers compared to moderate smokers' THC  $C_{\text{max}}$  following vaporization of 500 mg 6.7% THC ground cannabis.<sup>[13]</sup> Differences in THC  $C_{\text{max}}$  after vaporization in this study could be due to differences in smoking history, inhalation topography, and titration. Additionally, less efficient cannabinoid vaporization can occur with increased plant material,<sup>[32]</sup> as we vaporized  $\sim 750 \text{ mg}$  ground cannabis compared to 500 mg in Hartman *et al.* Although no statistically significant differences in  $C_{\text{max}}$  between smoking and vaporization were observed, differences in heating temperature could potentially



**Figure 5.**  $\Delta^9$ -tetrahydrocannabinol (THCV), cannabidiol (CBD), and cannabigerol (CBG) detection rates (%) up to 26 h at the proposed cut-offs (0.3, 0.5, and 0.3 µg/L, respectively) for  $n=11$  frequent (left) and  $n=9$  occasional (right) smokers after smoked, vaporized, and oral cannabis (6.9%  $\Delta^9$ -tetrahydrocannabinol, THC; ~50.6 mg THC) administration (0 h).

release fewer cannabinoids during vaporization compared to smoking, although less pyrolysis of THC would be expected and there is no loss of THC in side-stream smoke as occurs during smoking. In addition, vaporization stores THC vapour in a plastic bag during heating, possibly losing small amounts of THC through absorption to the bag.

Frequent and occasional smokers' THC  $C_{\max}$  following oral consumption were not significantly different, as this route is not amenable to self-titration. Our mean THC  $C_{\max}$  were lower than those reported by Vandrey *et al.* following 25 or 50 mg oral (brownie) THC in drug-free users ( $n=6$ ) but were more similar to those reported for 10 mg oral THC in the same study.<sup>[21]</sup> Observed differences in oral THC concentrations could be due to our later collection time (0.17 h post-inhalation dose, equating to 0.33 h post-oral dose compared to their 0.2 h time point). In a separate study, Niedbala *et al.* reported 2.2–7.1 µg/L THC 1–2 h after oral brownie consumption (20–25 mg THC) in casual users ( $n=3$ ).<sup>[17]</sup> By 1.5 h post-dose in our study, THC concentrations after oral cannabis were much greater (10.8–938 and 23.0–256 µg/L in frequent and occasional smokers, respectively) than Niedbala's reported THC concentrations at the same time post-dose; this may be due to a combination of different brownie preparations (i.e., how well the precursor  $\Delta^9$ -tetrahydrocannabinolic acid is converted to THC during baking) and our higher-potency THC variety. Compared to oral synthetic THC (dronabinol, Marinol®) administration, we observed increases in THC OF concentrations post-dose due to oral cavity

contamination that did not occur with encapsulated synthetic oral THC (dronabinol).<sup>[33,34]</sup>

THC and CBG exhibited significantly higher concentrations in frequent smokers' OF after inhaled routes than after oral cannabis dosing. Smoked and vaporized cannabis administration were previously reported to produce similar cannabinoid OF concentrations<sup>[13]</sup>; this was observed, except for a later CBG  $t_{\text{last}}$  after smoked cannabis compared to the vaporized dose. However, due to the greater CBG concentrations after smoked compared to vaporized cannabis, this could be expected. The same trend was observed with CBG disposition in whole blood and could be explained by inefficient CBG volatilization during vaporization. Significantly later  $t_{\text{last}}$  for minor cannabinoids in frequent smokers after inhaled cannabis could be due to the much greater concentrations achieved compared to brownie consumption. Differences in  $C_{\max}$  between the routes were not anticipated amongst occasional smokers as they often exhibit inefficient smoking/vaporization topography, leading to lower cannabinoid concentrations after inhalation similar to those following oral administration. Lower OF THC concentrations achieved after oral cannabis compared to inhaled cannabis could be due to oral intake mechanism (chewing and swallowing may not release as much THC as inhaling), ad libitum study design, conversion to CBN, degradation during baking, or possibly less efficient decarboxylation of acid to THC.

Cannabinoid metabolite detection and interpretation in OF can be complicated. 11-OH-THC is detected infrequently and only for

a short period of time. While detection of 11-OH-THC in oral fluid is an indication of recent cannabis use, its absence does not preclude recent consumption. OF THCCOOH concentration variability observed in this study was also previously observed.<sup>[13,15,16]</sup>

For the first time, THCV and CBG disposition were characterized in OF for frequent and occasional smokers following controlled smoked, vaporized, and oral (brownie) cannabis consumption with similar detection rates between groups and routes. Previously, THCV and CBG disposition were only described in urine.<sup>[35–37]</sup> Mean THCV  $C_{\max}$  ( $t_{\max}$ ) were 17.5–40.2  $\mu\text{g/L}$  (0.17–0.29 h) after inhalation routes and 3.2–4.5  $\mu\text{g/L}$  (0.47–0.53 h) after oral dosing among all participants, while mean CBG  $C_{\max}$  ( $t_{\max}$ ) were 87.4–244  $\mu\text{g/L}$  (0.17 h) after inhaled routes and 11.9–17.0  $\mu\text{g/L}$  (0.41–0.47 h) after oral dosing for all participants. In our cohort, both THCV and CBG were detected in 11/11 frequent and 7/9 occasional (THCV) and 11/11 frequent and 9/9 occasional (CBG) smokers after all administration routes for up to 26 h at the LOQ of 0.2  $\mu\text{g/L}$ , making them applicable for identifying cannabis intake within about one day, as previously suggested by Desrosiers *et al.*<sup>[26]</sup> THCV was not detected in two occasional smokers after vaporization with low THC concentrations (7.5 and 8.5  $\mu\text{g/L}$  at 0.17 h). CBD was previously investigated in OF following smoked<sup>[15]</sup> and vaporized<sup>[13]</sup> cannabis administration in different cohorts. While CBD  $C_{\max}$  after smoking in this study (0.17–0.29 h) were slightly higher than those observed in frequent and occasional smokers 0.5 h after smoking,<sup>[15]</sup> our median occasional smokers' CBD  $C_{\max}$  after vaporization were comparable to those reported for moderate smokers after the high THC dose without alcohol in Hartman *et al.* vaporization study.<sup>[13]</sup> To our knowledge, CBD was not previously investigated in OF following oral cannabis administration. CBD, while a useful marker of recent use in this study, cannot be thoroughly characterized until investigated at the higher-potency CBD cannabis material now available in the market.

DRUID and SAMHSA established THC OF confirmation cut-off guidelines of 1 and 2  $\mu\text{g/L}$ , respectively. However, frequent smokers' THC concentrations remain well above these cut-offs for longer periods of time, making data interpretation difficult for estimating recent use. Occasional smokers' OF THC concentrations are generally lower and may fall below the DRUID or SAMHSA cut-offs within a much shorter timeframe, making it difficult to capture recent use beyond several hours. OF THC concentrations after oral cannabis consumption are not as high as concentrations observed following inhaled routes and concentrations fall below DRUID and SAMHSA cut-offs much more quickly. Additionally, THC peak concentrations in OF following edible cannabis consumption occur prior to peak impairment, with no secondary peak after oral consumption, suggesting oral mucosa contamination rather than partitioning from blood.

In order to establish detection windows for capturing recent cannabis use, we previously investigated several combinations of cut-offs, including THC in combination with CBD, CBN, and/or THCCOOH.<sup>[13,15,16]</sup> Our new expanded OF cannabinoid method incorporates THCV and CBG as additional analytes, although CBN could no longer be included as we could not chromatographically separate it from a matrix interference. Our low LOQs for minor cannabinoids, including THCV and CBG, allowed us to detect these analytes up to 26 h following controlled cannabis administration. By applying a 0.3  $\mu\text{g/L}$  cut-off for THCV or CBG, detection windows were 8 and 20 h, respectively, for both frequent and occasional smokers. Monitoring minor cannabinoids in OF offers the ability to detect recent cannabis use that is not achievable for THC and/or THCCOOH. However, minor cannabinoids (THCV, CBN,

CBD, and CBG) were not detected in whole blood specimens collected from the same cohort following oral brownie administration (manuscript under review). While these data offer promising results for capturing recent cannabis use by increasing the cannabinoids analyzed in OF specimens, more research is necessary following other administration routes, including vape pens, dabs, waxes, THC oils, and other cannabis and/or THC products. Comparison to pharmacodynamic outcomes as well as on-site OF screening devices and other matrices would assist in further interpretation of OF cannabinoid data.

## Conclusions

THC, metabolites, and minor cannabinoids were fully characterized in OF following controlled cannabis brownie consumption with direct comparison to smoked and vaporized administration in the same frequent and occasional smokers. The within-subject study design allowed for direct pharmacokinetic comparisons between the different administration routes. Few differences were observed between smoked and vaporized cannabis administrations. For the first time, THCV and CBG were characterized in OF after multiple routes and CBD was characterized for the first time after oral cannabis administration. Cannabinoid concentrations peaked immediately after cannabis consumption, regardless of route, as a result of oral mucosa contamination. As expected, greater THC concentrations were observed after smoked and vaporized cannabis compared to oral administration. Minor cannabinoids, including THCV, CBD, and CBG, were detected in 18/20, 20/20, and 20/20 study participants, respectively, after all three administration routes and up to 26 h post-dose, indicating potential utilization as markers of cannabis use within 1 day that could be helpful in interpretation of clinical and forensic drug testing programmes.

## Acknowledgements

The authors would like to thank Dr Sandrine Pirard for her contribution to study design and the contributions of the clinical staffs of the Intramural Research Program, National Institute on Drug Abuse, and the Clinical Research Unit, Johns Hopkins Bayview Medical Center. This study was registered on clinicaltrials.gov (NCT02177513). Quantisal devices and BG100  $\beta$ -glucuronidase were provided by the manufacturers to NIH via Materials Transfer Agreements. This research was funded by the Intramural Research Program, National Institute on Drug Abuse, NIH. MNN acknowledges the Graduate Partnership Program, NIH.

## References

- [1] United Nations. *World Drug Report 2015*, United Nations, Vienna, **2015**.
- [2] A. Berning, R. Compton, K. Wochinger. Results of the 2013–2014 National Roadside Survey of Alcohol and Drug Use by Drivers. *Traffic Safety Facts: Research Note*. National Highway Traffic Safety Administration, Washington, DC, **2015**.
- [3] M. Asbridge, R. Mann, M. D. Cusimano, C. Trayling, M. Roerecke, J. M. Tallon, A. Whipp, J. Rehm. Cannabis and traffic collision risk: findings from a case-crossover study of injured drivers presenting to emergency departments. *Int. J. Public Health*. **2014**, 59, 395.
- [4] M.-C. Li, J. E. Brady, C. J. DiMaggio, A. R. Lusardi, K. Y. Tzong, G. Li. Marijuana use and motor vehicle crashes. *Epidemiol. Rev.* **2011**, 34, 65.
- [5] J. G. Ramaekers, G. Berghaus, M. van Laar, O. H. Drummer. Dose related risk of motor vehicle crashes after cannabis use. *Drug Alcohol Depend.* **2004**, 73, 109.



- [6] R. L. Hartman, M. A. Huestis. Cannabis effects on driving skills. *Clin. Chem.* **2013**, 59, 478.
- [7] T. Van der Linden, S. A. Legrand, P. Silverans, A. G. Verstraete. DUID: oral fluid and blood confirmation compared in Belgium. *J. Anal. Toxicol.* **2012**, 36, 418.
- [8] N. Samyn, G. De Boeck, A. G. Verstraete. The use of oral fluid and sweat wipes for the detection of drugs of abuse in drivers. *J. Forensic Sci.* **2002**, 47, 1380.
- [9] S. Strano-Rossi, E. Castrignano, L. Anzillotti, G. Serpelloni, R. Mollica, F. Tagliaro, J. P. Pascali, D. di Stefano, R. Sgalla, M. Chiarotti. Evaluation of four oral fluid devices (DDS®, Drugtest 5000®, Drugwipe 5+® and RapidSTAT®) for on-site monitoring drugged driving in comparison with UHPLC-MS/MS analysis. *Forensic Sci. Int.* **2012**, 221, 70.
- [10] T. Blencowe, A. Pehrsson, P. Lillsunde, K. Vimpari, S. Houwing, B. Smink, R. Mathijssen, T. Van der Linden, S. A. Legrand, K. Pil, A. Verstraete. An analytical evaluation of eight on-site oral fluid drug screening devices using laboratory confirmation results from oral fluid. *Forensic Sci. Int.* **2011**, 208, 173.
- [11] V. Vindenes, H. M. Lund, W. Andresen, H. Gjerde, S. E. Ikdahl, A. S. Christophersen, E. L. Øiestad. Detection of drugs of abuse in simultaneously collected oral fluid, urine and blood from Norwegian drug drivers. *Forensic Sci. Int.* **2012**, 219, 165.
- [12] M. Concheiro, A. de Castro, O. Quintela, A. Cruz, M. López-Rivadulla. Confirmation by LC-MS of drugs in oral fluid obtained from roadside testing. *Forensic Sci. Int.* **2007**, 170, 156.
- [13] R. Hartman, S. Anizan, M. Jang, T. L. Brown, K. Yun, D. Gorelick, G. Milavetz, A. Spurgin, G. Gaffney, M. Huestis. Cannabinoid disposition in oral fluid after controlled vaporizer administration with and without alcohol. *Forensic Toxicol.* **2015**.
- [14] G. L. Schauer, B. A. King, R. E. Bunnell, G. Promoff, T. A. McAfee. Toking, vaping, and eating for health or fun: marijuana use patterns in adults, USA, 2014. *Am. J. Prev. Med.* **2016**, 50, 1.
- [15] S. Anizan, G. Milman, N. Desrosiers, A. J. Barnes, D. A. Gorelick, M. A. Huestis. Oral fluid cannabinoid concentrations following controlled smoked cannabis in chronic frequent and occasional smokers. *Anal. Bioanal. Chem.* **2013**, 405, 8451.
- [16] M. N. Newmeyer, N. A. Desrosiers, D. Lee, D. R. Mendu, A. J. Barnes, D. A. Gorelick, M. A. Huestis. Cannabinoid disposition in oral fluid after controlled cannabis smoking in frequent and occasional smokers. *Drug Test. Anal.* **2014**, 6, 1002.
- [17] R. Niedbala, K. Kardos, D. Fritch, S. Kardos, T. Fries, J. Waga, J. Robb, E. Cone. Detection of marijuana use by oral fluid and urine analysis following single-dose administration of smoked and oral marijuana. *J. Anal. Toxicol.* **2001**, 25, 289.
- [18] S. Toennes, J. Ramaekers, E. Theunissen, M. Moeller, G. Kauert. Pharmacokinetic properties of  $\Delta^9$ -tetrahydrocannabinol in oral fluid of occasional and chronic users. *J. Anal. Toxicol.* **2010**, 34, 216.
- [19] D. Lee, D. M. Schwoppe, G. Milman, A. J. Barnes, D. A. Gorelick, M. A. Huestis. Cannabinoid disposition in oral fluid after controlled smoked cannabis. *Clin. Chem.* **2012**, 58, 748.
- [20] D. Lee, R. Vandrey, D. R. Mendu, J. A. Murray, A. J. Barnes, M. A. Huestis. Oral fluid cannabinoids in chronic frequent cannabis smokers during ad libitum cannabis smoking. *Drug Test. Anal.* **2015**, 7, 494.
- [21] J. M. Mitchell, E. J. Cone, R. Flegel, C. LoDico, E. S. Herrmann, G. E. Bigelow, R. Vandrey. Oral administration of cannabis in brownies. Society of Forensic Toxicologists Annual Meeting, Atlanta, GA, USA, **2015**.
- [22] H. Schulze, M. Schumacher, R. Urmeew, K. Auerbach, J. Alvarez, I. M. Bernhoft, H. de Gier, M. Hagenzieker, S. Houwing, A. Knoche, M. Pilgerstorfer, B. Zlender. Driving Under the Influence of Drugs, Alcohol and Medicines in Europe – finding from the DRUID project. *EMCDDA*, Lisbon, **2012**.
- [23] S. A. M. H. S. Administration. Mandatory Guidelines for Federal Workplace Drug Testing Programs. Federal Register **2015**, 80 28054.
- [24] D. Lee, G. Milman, D. M. Schwoppe, A. J. Barnes, D. A. Gorelick, M. A. Huestis. Cannabinoid stability in authentic oral fluid after controlled cannabis smoking. *Clin. Chem.* **2012**, 58, 1101.
- [25] S. Anizan, M. M. Bergamaschi, A. J. Barnes, G. Milman, N. Desrosiers, D. Lee, D. A. Gorelick, M. A. Huestis. Impact of oral fluid collection device on cannabinoid stability following smoked cannabis. *Drug Test. Anal.* **2014**, 7, 114.
- [26] N. A. Desrosiers, K. B. Scheidweiler, M. A. Huestis. Quantification of six cannabinoids and metabolites in oral fluid by liquid chromatography-tandem mass spectrometry. *Drug Test. Anal.* **2015**, 7, 684.
- [27] C. Moore, S. Rana, C. Coulter, D. Day, M. Vincent, J. Soares. Detection of conjugated 11-nor-Delta9-tetrahydrocannabinol-9-carboxylic acid in oral fluid. *J. Anal. Toxicol.* **2007**, 31, 187.
- [28] M. Vondracek, Z. Xi, P. Larsson, V. Baker, K. Mace, A. Pfeifer, H. Tjälve, M. T. Donato, M. J. Gomez-Lechon, R. C. Grafström. Cytochrome P450 expression and related metabolism in human buccal mucosa. *Carcinogenesis*. **2001**, 22, 481.
- [29] S.-P. Yang, G. M. Raner. Cytochrome P450 expression and activities in human tongue cells and their modulation by green tea extract. *Toxicol. Appl. Pharmacol.* **2005**, 202, 140.
- [30] P. G. Sacks, Z.-L. Zhao, W. Kosinska, K. E. Fleisher, T. Gordon, J. B. Guttenplan. Concentration dependent effects of tobacco particulates from different types of cigarettes on expression of drug metabolizing proteins, and benzo(a)pyrene metabolism in primary normal human oral epithelial cells. *Food Chem. Toxicol.* **2011**, 49, 2348.
- [31] L. X. Zhou, B. Pihlstrom, J. P. Hardwick, S. S. Park, S. A. Wrighton, J. L. Holtzman. Metabolism of phenytoin by the gingiva of normal humans: the possible role of reactive metabolites of phenytoin in the initiation of gingival hyperplasia. *Clin. Pharmacol. Ther.* **1996**, 60, 191.
- [32] A. Hazekamp, R. Ruhaak, L. Zuurman, J. van Gerven, R. Verpoorte. Evaluation of a vaporizing device (Volcano®) for the pulmonary administration of tetrahydrocannabinol. *J. Pharm. Sci.* **2006**, 95, 1308.
- [33] G. Milman, A. Barnes, D. Schwoppe, E. Schwilke, R. Goodwin, D. Kelly, D. Gorelick, M. Huestis. Cannabinoids and metabolites in expectorated oral fluid after 8 days of controlled around-the-clock oral THC administration. *Anal. Bioanal. Chem.* **2011**, 401, 599.
- [34] G. Milman, A. J. Barnes, D. M. Schwoppe, E. W. Schwilke, W. D. Darwin, R. S. Goodwin, D. L. Kelly, D. A. Gorelick, M. A. Huestis. Disposition of cannabinoids in oral fluid after controlled around-the-clock oral THC administration. *Clin. Chem.* **2010**, 56, 1261.
- [35] E. Hidvegi, G. P. Somogyi. Detection of cannabigerol and its presumptive metabolite in human urine after Cannabis consumption. *Pharmazie*. **2010**, 65, 408.
- [36] M. A. ElSohly, S. Feng, T. P. Murphy, A. W. Warrington, S. Ross, A. Nimrod, Z. Mehmedic, N. Fortner. Identification and quantitation of 11-nor-delta-tetrahydrocannabivarin-9-carboxylic acid, a major metabolite of delta-tetrahydrocannabivarin. *J. Anal. Toxicol.* **2001**, 25, 476.
- [37] F. R. Levin, J. J. Mariani, D. J. Brooks, S. Xie, K. A. Murray.  $\Delta^9$ -Tetrahydrocannabivarin testing may not have the sensitivity to detect marijuana use among individuals ingesting dronabinol. *Drug Alcohol Depend.* **2010**, 106, 65.